The genetic basis of dyslexia:

The *KIAA0319* gene

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Summary

- Overview about the genetics of dyslexia
- Focus on the chromosome 6p locus and the KIAA0319 candidate: the gene and the protein
- The role of the dyslexia candidates in brain development

Dyslexia-definition

- Specific difficulty in learning to read that cannot be explained by deficits in intelligence, learning opportunity or any evident neurological or sensory handicap.

- Reading ability is a continuous measure; there is no an universally accepted threshold to classify an individual for being affected.

Reading

Two component processes:

- Phonological Processing
  - Breakdown of words into their constituent phonemes or speech sounds through the use of a set of pronunciation rules
    - AUTOMATIC "Ah-toe-Mah-tik"

- Orthographic Processing
  - Holistic recognition of words based on the memorised spatial appearance of letters. Requires previous word exposure
    - YACHT

Dyslexia - genetic component

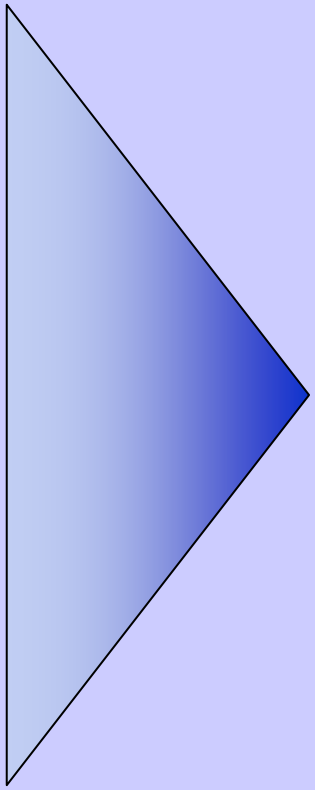
- Risk in population: 5-10%
- Male / famale : 3/2
- Risk in sibling of affected individuals: 38-43%
- Twin studies
  - Concordance rate: 68%in MZ versus 38%in DZ
- Complex trait, influenced by both environmental and multiple genetic factors (quantitative trait loci = QTL)

Theories of dyslexia

The biological and cognitive causes underlying the development of dyslexia are not clear.

Several theories have been proposed:

- Phonological deficit
- Auditory processing
- Visual processing
- Motor control
- Magnocellular theory

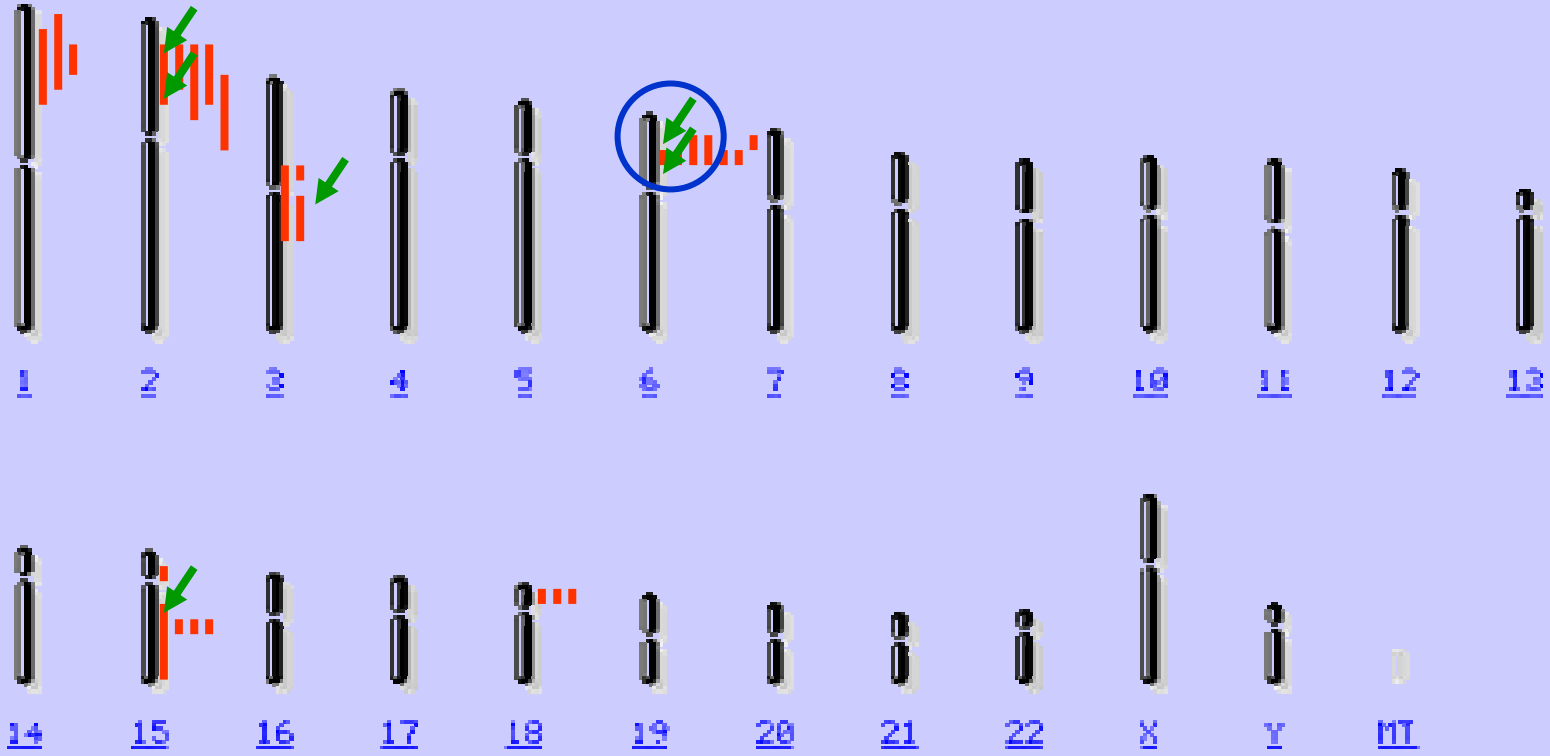


We expect the dyslexia susceptibility genes to be expressed in the brain but we don't have a functional model

Overview of genetic analysis results

Regions identified by linkage analysis that might contain QTLs for dyslexia susceptibility

Candidate genes identified by association analysis or translocation breakpoint refinement



Our Dyslexia Samples

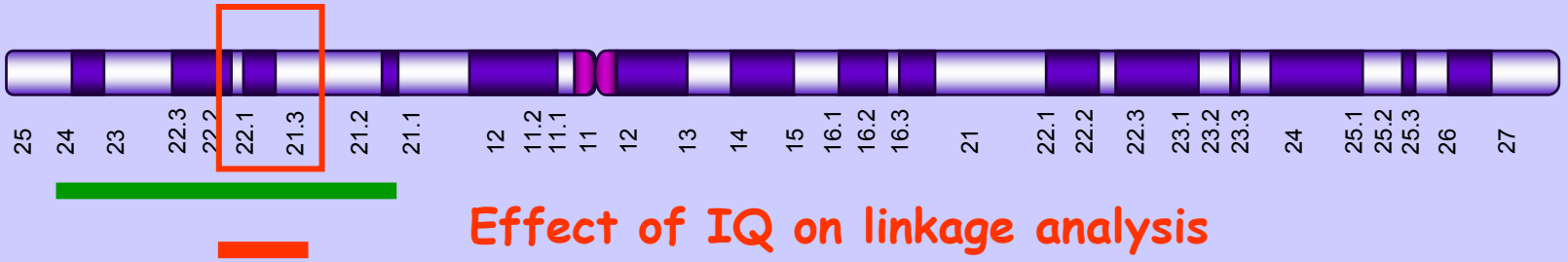
- **Genetic samples from 264 nuclear UK families:**
  - Divided in sample 1 (89 families) and sample 2 (175 families)
  - All contain at least one dyslexic child (scoring on single-word reading test at least 2 SD below what predicted by test of verbal and non-verbal reasoning).
  - At least 68% contain another child with reading-related problems.
  - Total of 1153 individuals:
    - ⇨ including 630 siblings measured for reading and language related quantitative traits.
- **Genetic samples from 155 twin-based nuclear US families from the CLDRC:**
  - Families selected for having at least one member with documented reading difficulty.
  - Total of 675 individuals:
    - ⇨ including 365 siblings measured for reading and language related quantitative traits.

Our sample -Quantitative phenotype

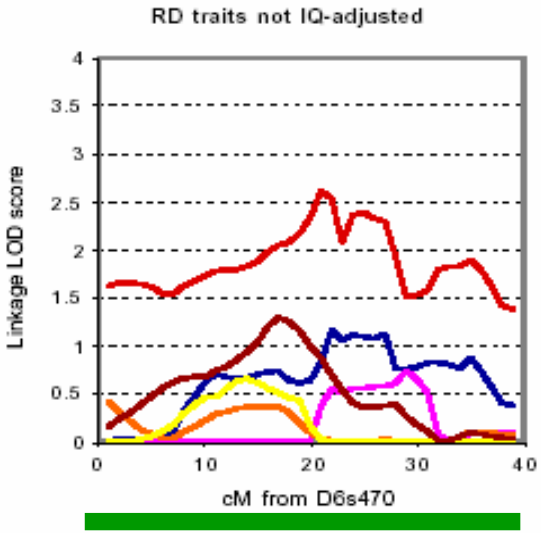
- Essentially 6 core traits tested for:
  - Orthographic coding: Irregular words recognition - OC-irreg
    - Example: Yacht
  - Phonological Decoding: Non-word recognition – PD
    - Example: Siglop
  - Orthographic coding: Forced Choice - OC-choice
    - Example: Rain versus rane
  - Word reading - READ
  - Spelling ability - SPELL
  - Phonological Awareness - PA (Spoonerisms)
    - Example: lazy dog -> daisy log
- High correlation between measures: 0.3-0.8

Chromososme 6

linkage analysis in 89 UK families, 224 siblings - SAMPLE 1



Effect of IQ on linkage analysis

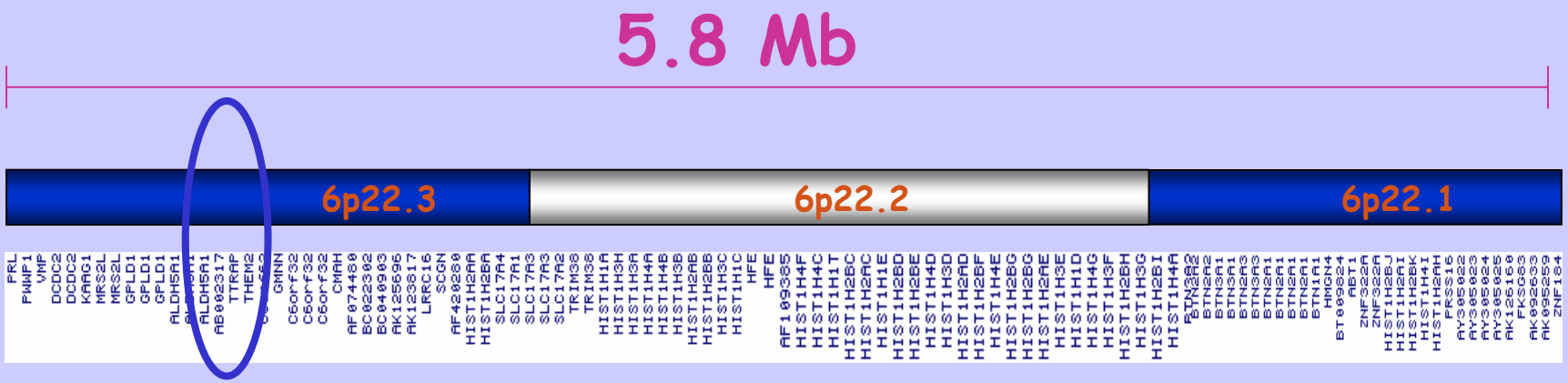


6 quantitative reading-related measures

- PD
- OC-irreg
- OC-choice
- Spell
- Read
- PA

Francks et al. AJHG 2004

Initial SNP association analysis



P = 0.0004

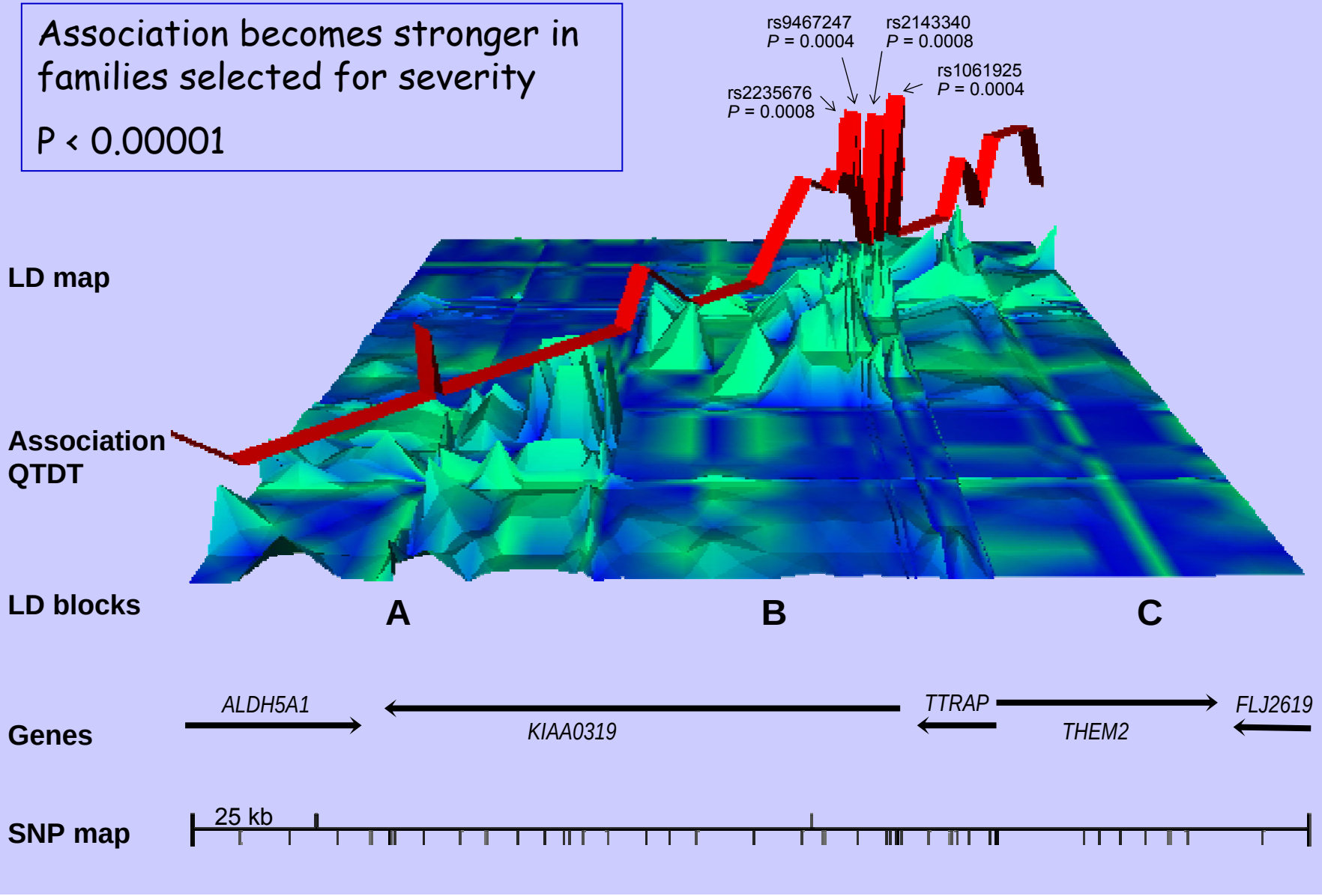
OC-irreg IQ adjusted

Statistical analysis by QTDT - Test of association for quantitative traits in nuclear families

Association analysis and LD evaluation in 89 UK families

Association becomes stronger in families selected for severity

P < 0.00001



Association *P*-values in the UK and US most severe cases

		UK SAMPLE 313 siblings, 126 families							US SAMPLE 310 siblings, 131 families					
Marker	LD region	Risk allele	OC-irreg	OC-choice	PD	READ	SPELL	PA	Risk allele	OC-choice	PD	READ	SPELL	PA
rs699463	A	1	0.0032		0.0231	0.0279	0.0153	0.0112						
rs4504469	B	1	0.0011		0.0082	0.004		0.01						
rs2179515	B	1	0.0012		0.0131	0.0004	0.0232							
rs761101	B	1	0.0025		0.0057	0.0006	0.0325							
rs6456624	B	1	0.0005		0.0045	0.0003	0.0157							
rs2328846	B	1	0.0007		0.0017	0.0003	0.0155							
rs2235676	B	2	0.0023	0.0009		0.0041			2			0.0127		
rs2038137	B	1	0.0013		0.0026	0.0002	0.0061							
k_pr_del	B	1	0.0011		0.0032	0.0002	0.0086							
k_pr_1	B	2	0.0006	0.0003	0.0373	0.0003	0.0016		2			0.0022		0.0446
rs1555090	B	1	0.001		0.0029	0.0003	0.0131							
rs3033236	B	2	0.0134	0.0104		0.0073			2		0.0295	0.0014	0.0090	0.0252
rs2143340	B	2	0.01	0.0003		0.0115			2		0.0404	0.0032	0.0141	0.0102
rs1061925	B	2	0.0009	0.0005		0.0008			2			0.0040	0.0256	
tt_th_del	C								2					0.0182
rs926529	C	1			0.0132									
rs1885211	C													
th_ex_3	C													
rs3756814	C	2	0.0332											
rs6456632	C	1				0.0415								

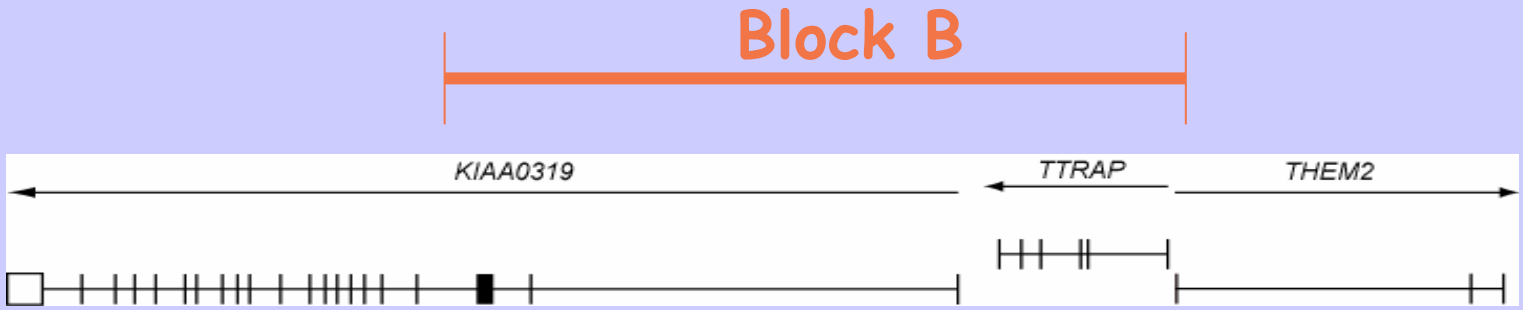
Block B

Colour-coded *P*-values:

- $0.01 < P < 0.05$
- $0.001 < P < 0.01$
- $P < 0.001$

A study in a completely independent sample detected association with 2 SNPs located in block B (AJHG Apr 2005)

Haplotype analysis

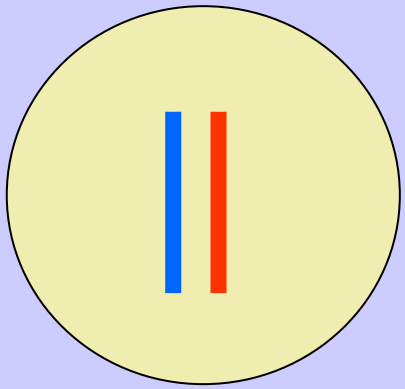


Tagging SNPs		rs4504469	rs2038137	rs2143340	UK %	UK $P_{(read)}$	US %	US $P_{(read)}$
Haplotypes	(1)	1	1	1	41		46	
	(2)	2	2	1	36		31	
	(3)	1	1	2	12	0.002	12	0.01
	(4)	2	1	1	5		5	
	(5)	1	2	1	4		4	

MUTATION SCREENING DID NOT REVEALED ANY OBVIOUS DISRUPTIVE MUTATION

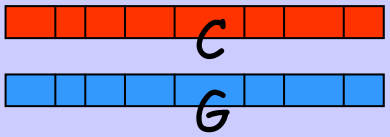
Allele-specific quantitative gene expression assay

Select cell lines heterozygous for the risk haplotype



Measure of relative quantitative differences in gene expression using:

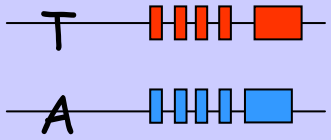
Coding SNPs



Make cDNA

PCR

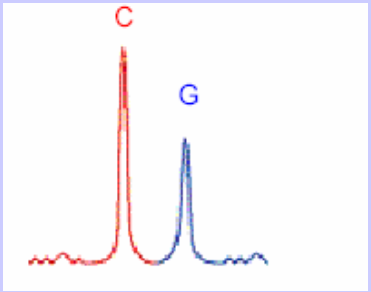
SNPs at promoter site



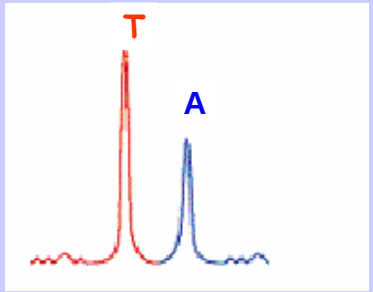
Immunoprecipitate chromatin (HaploChIP)

PCR

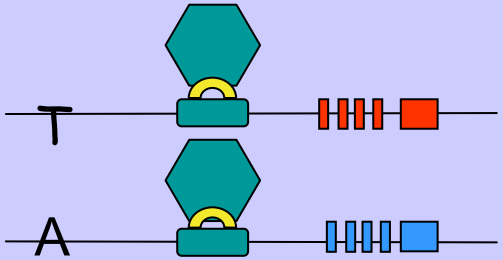
Primer extension
Mass spectrometry



Measure of peak areas is proportional to relative abundance of the 2 alleles



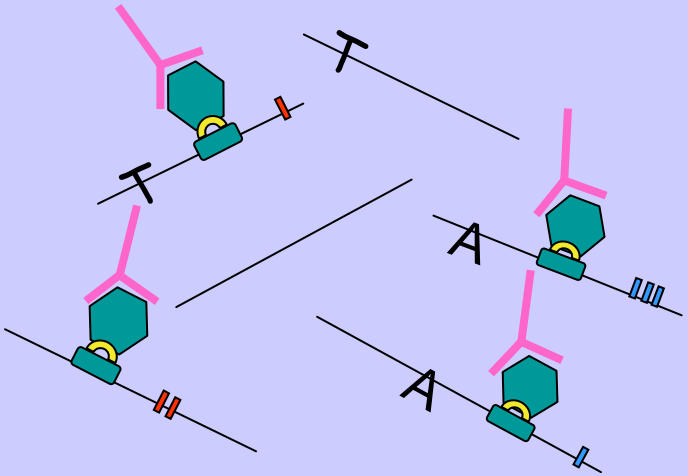
HaploChIP principle



SNP RNA Pol II binding site

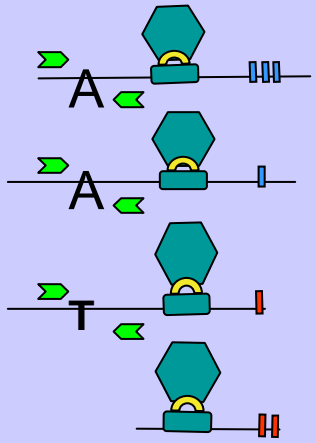
Protein-DNA crosslinking *in vivo*

Sonication



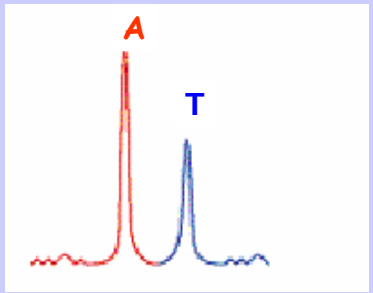
Immunoprecipitation with antibody specific to RNA Pol II

Protein digestion



PCR and allele quantification

THE RELATIVE QUANTITY OF THE 2 ALLELES IS A MEASURE OF RNA POL II AFFINITY TO THE 2 HAPLOTYPES

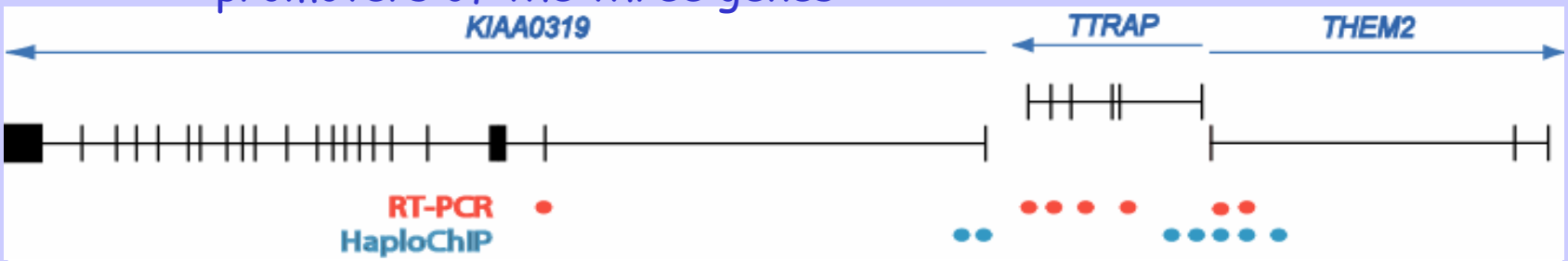


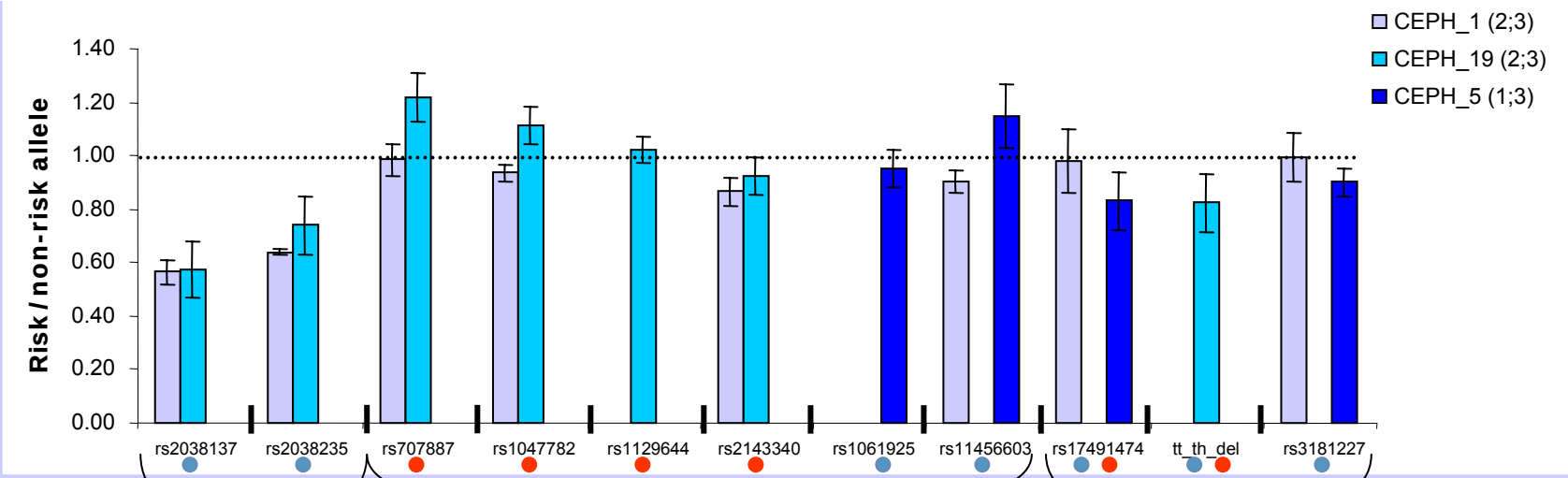
Nat Genet 2003, 33:469-75

Risk haplotype effect on gene expression

3 CEPH lymphoblastoid cell lines:

- Heterozygous for risk haplotype
- Heterozygous for SNPs within the transcripts or the promoters of the three genes

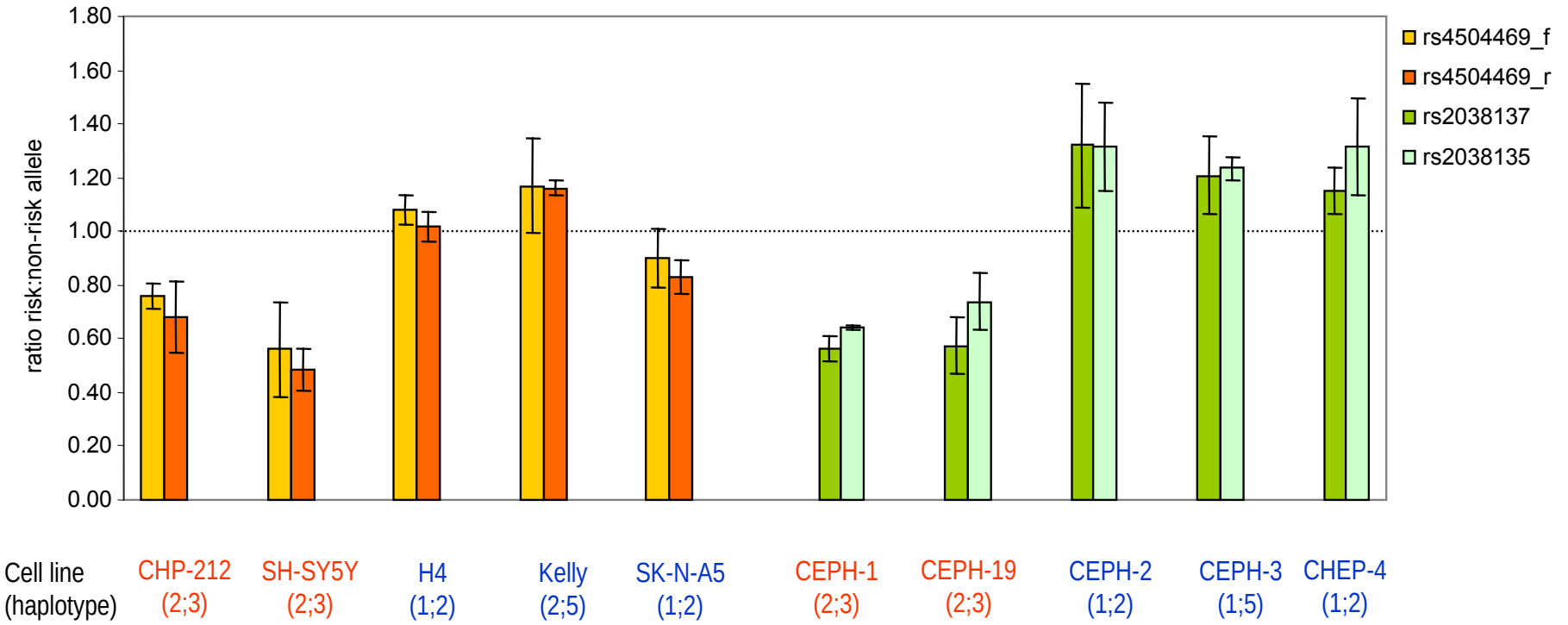




P < 0.0001

Paracchini et al, HMG 2006

The risk haplotype is associated to a reduced expression of the *KIAA0319* gene



Risk v non-risk
$P = 2 \times 10^{-7}$

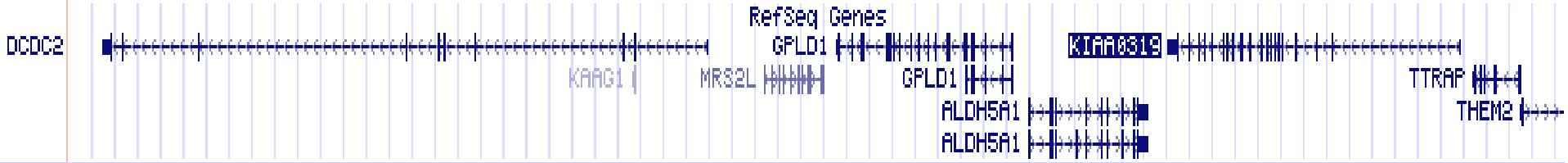
Risk v non-risk
$P = 7 \times 10^{-16}$

The *DCDC2* gene

Two studies found association within *DCDC2*, less than 200kb away from KIAA0319:

- Meng et al., 2005: Association with 2 SNPs + identification of an intronic deletion somehow link to dyslexia in 153 US families from CLDR

- Schumacher et al., 2006: Two-SNPs haplotype within intron seven associated in two independent German trio samples. Association is stronger in severe sub-groups



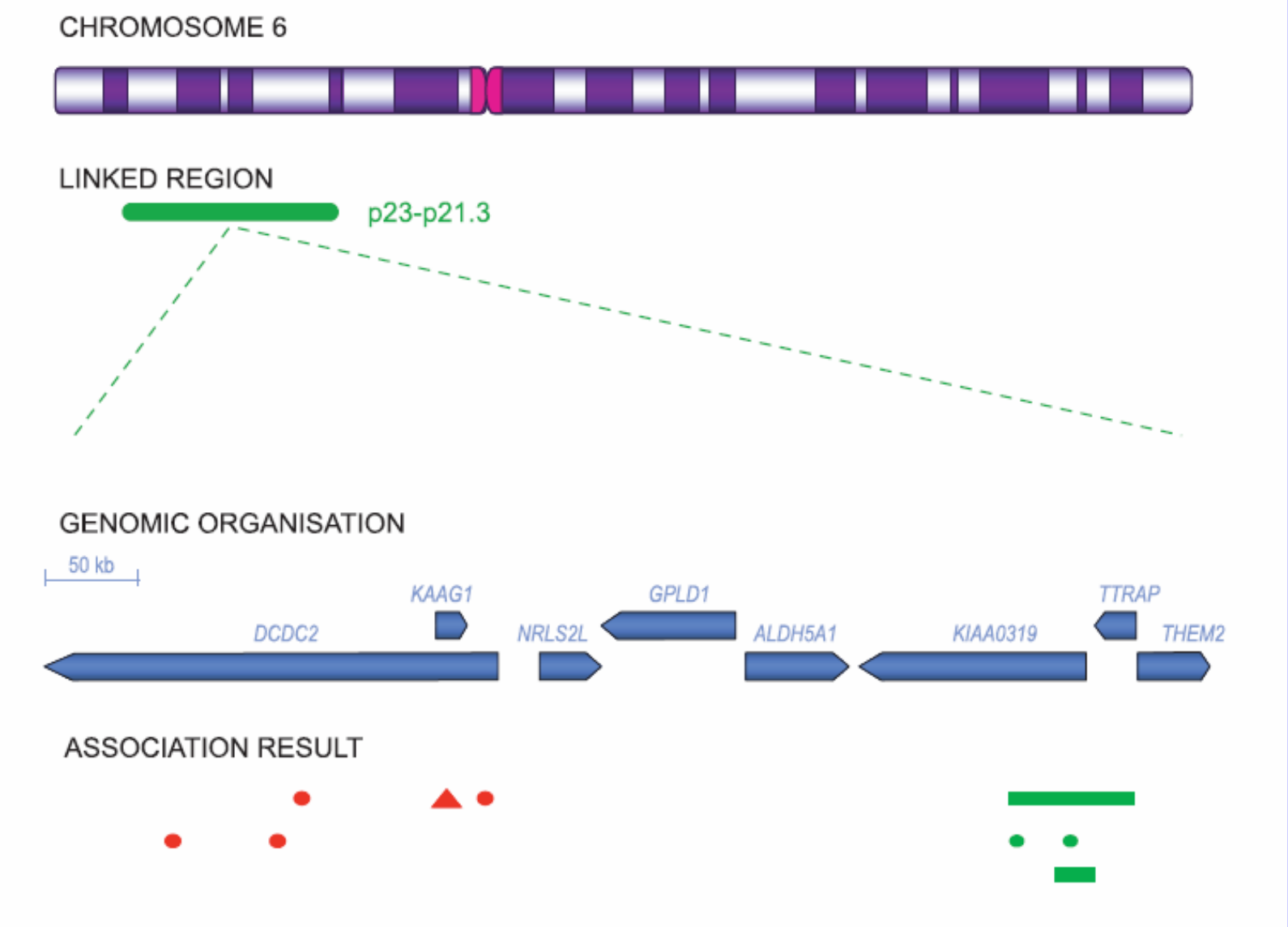
KIAA0319 v DCDC2

Oxford/Cardiff collaboration

Harold et al, Mol Psychiatry 2006

			Selected sibships from the Oxford sample n = 313 siblings, 126 families							Cardiff cases and controls n = 350 / n = 273	
			P value for trait								
Marker	Gene	Location	Risk allele[a]	OC-irreg	PD	OC-choice	READ	SPELL	PA	Risk allele[a]	P value
rs793862_rs807701	*DCDC2*	Intron 7									
rs793862	*DCDC2*	Intron 7									
rs807701	*DCDC2*	Intron 7									
rs807724	*DCDC2*	Intron 6									
DCDC2_deletion	*DCDC2*	Intron 3	2	0.0298			0.0428		0.0478		
rs1087266	*DCDC2*	Intron 2									

Chromosome 6p: result summary



Paracchini et al, ARGG in press

Chromosome 6p: result interpretation

- Associations are different signals of a unique causal mutations
- Different association are the results of different ascertainment and analysis criteria.
- Each gene contribute to a specific sub-group of dyslexia
- Definitive answer would come from the identification of the causal genetic variants

The Colorado case

Reference	Number of families	Selection criteria	Associated gene
Deffenbacher et al. (2004)	114	A sib with severe score in at least one trait	*DCDC2* *KIAA0319*
Francks et al. (2004)	126	A sib with severe score on a composite measure of traits contributing to the linkage	*KIAA0319*
Meng et al. (2005)	153	No selection	*DCDC2*

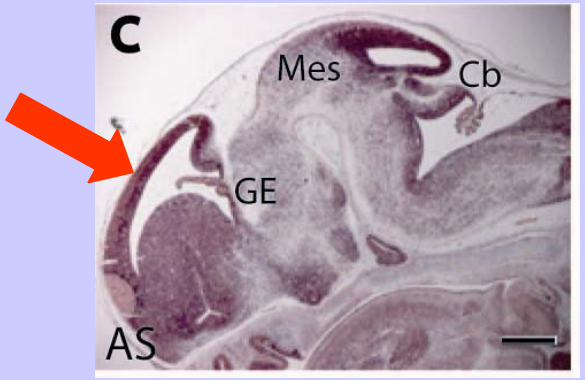
→ Each gene contribute to specific subgroup of dyslexia

→ The analysis is very sensitive to sample selection

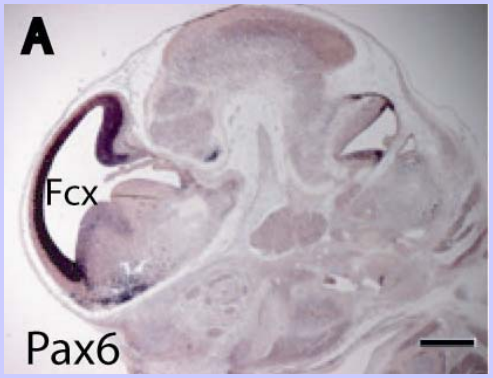
KIAA0319: In situ expression

Andy Copp, UCL

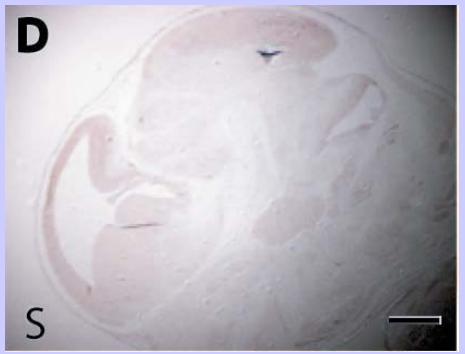
Mouse brain E13.5



KIAA0319

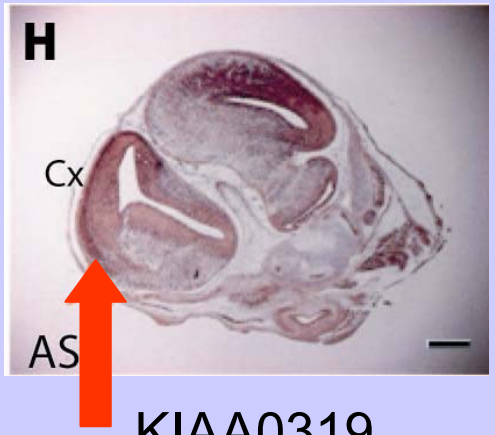


Positive control

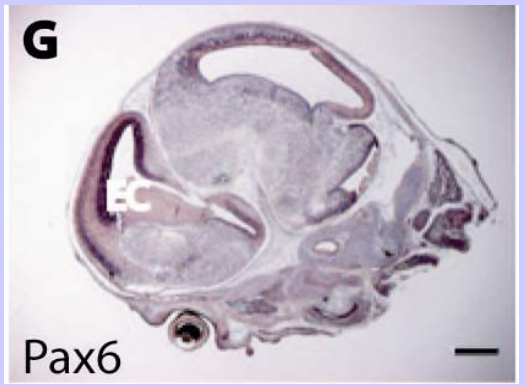


Negative control

Mouse brain E15.5



KIAA0319

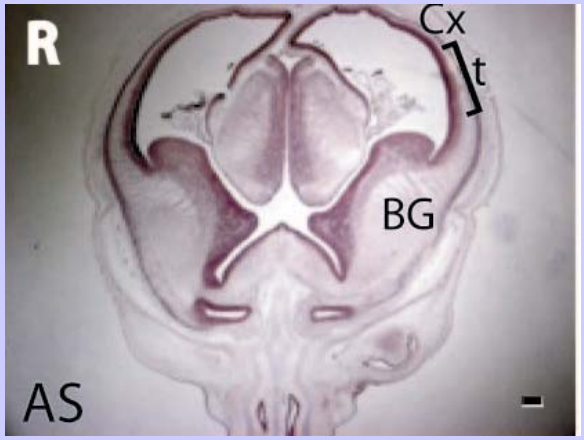


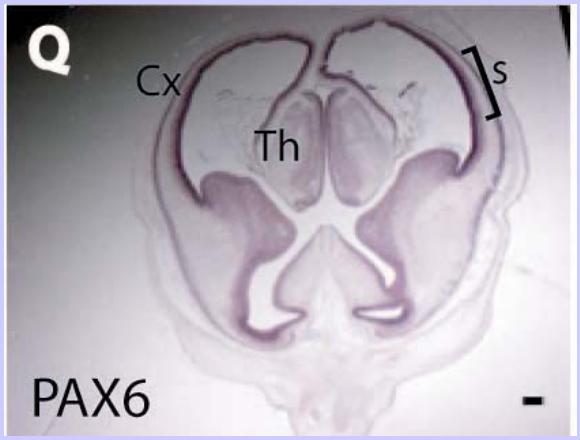
Positive control

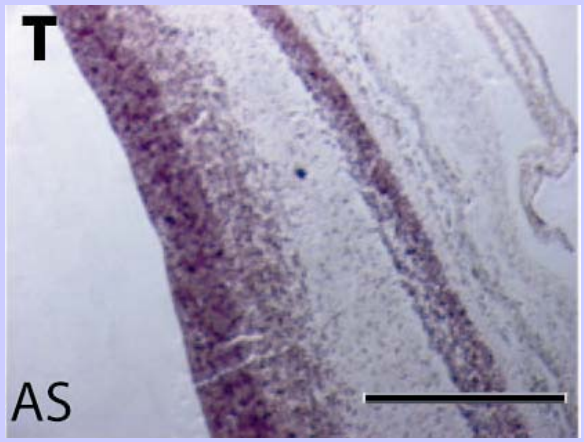
Paracchini et al, HMG 2006

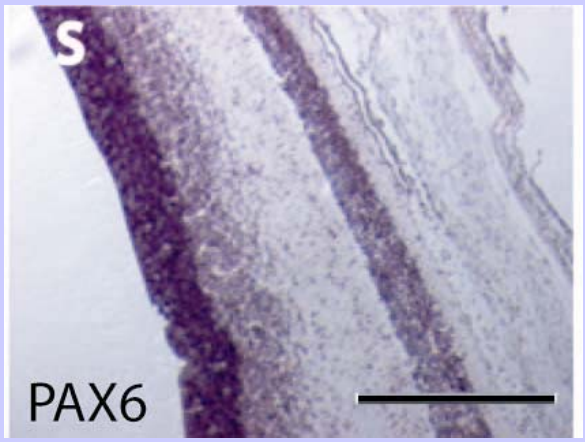
KIAA0319: In situ expression

Early fetal human brain





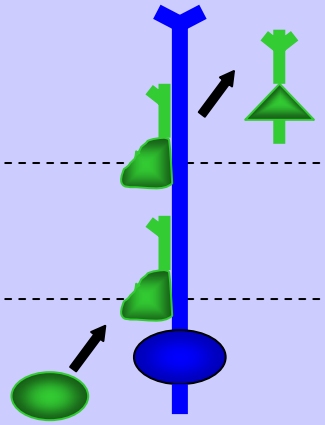




In utero RNA interference

Joe LoTurco, University of Connecticut

Glial-guided neuronal migration



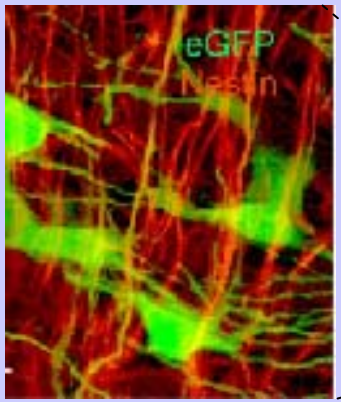
THEM2

TTRAP

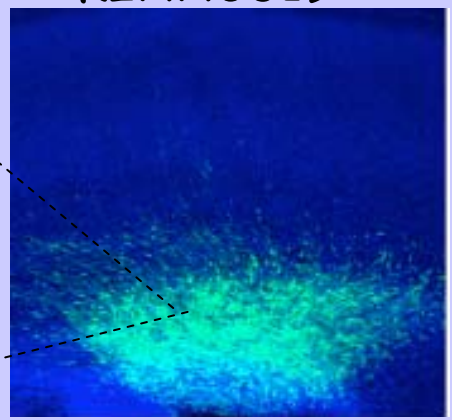
Cortical plate

Intermediate zone

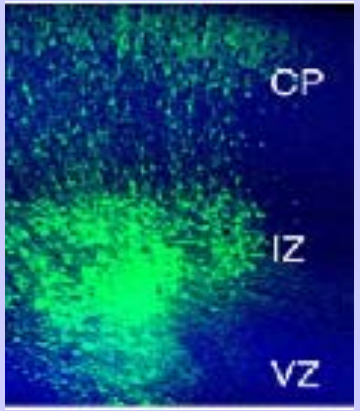
Ventricular zone



KIAA0319



KIAA0319 - rescue



Paracchini et al, HMG 2006

Neuronal migration and dyslexia

- Description of different dyslexic brains revealed cortical malformations mainly in the frontal region and in the left language areas consistent with abnormal neuronal migration (Galaburda's work):
  - Ectopias (small neuronal congregations in an abnormal layer location)
  - Dysplasia (loss of cortical neurons organisation)
- The neuronal migration disorder of periventricular nodular heterotopia has been found to be associated with an impairment in reading skills in presence of otherwise normal intelligence (Chang et al., 2005).

Neuronal migration and KIAA0319

- KIAA0319 is a transmembrane protein exposing PKD domains outside the cell.
- PKD domains have cell adhesion properties.
- It is possible that KIAA0319 is required for appropriate cell adhesion between migrating neurons and the glial fibers during the development of the neocortex.

Other candidate genes

Gene	Independent replications	Functional variants	Functional mechanism	Brain expression	Reference
KIAA0319	At least 3	NO	Gene expression	YES	Francks (2004) Cope (2005) Harold (2006) Paracchini (2006)
DCDC2	2	NO		YES	Meng (2005) Schumacher (2006)
DYX1C1	?	NO		YES	Taipale (2003)
ROBO1	None	NO	Gene expression	YES	Hannula-Jouppi (2005)
MRPL19	2 (same study)	NO	Gene expression	YES	Anthoni (2007)
C2ORF3	2 (same study)	NO	Gene expression	YES	Anthoni (2007)

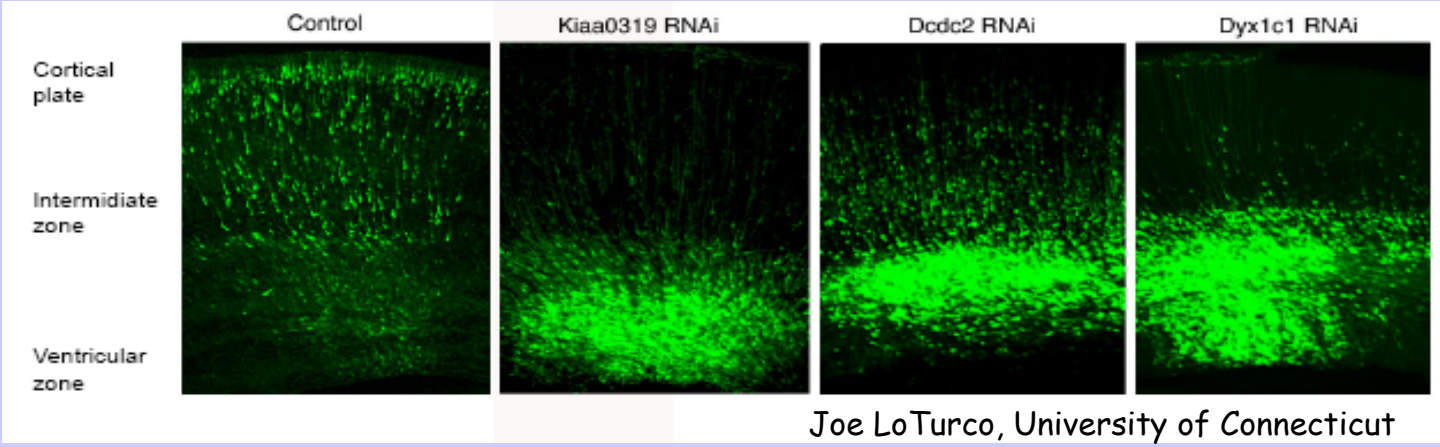
DYX1C1: replication attempts

Reference	Proband's disorder	Country of origin	Most significantly reported P-values[a] for individual SNPs or haplotypes within *DYX1C1*		
			-3G > A	1249G > T	-3G > A:1249G > T
Taipale et al. (95)	Dyslexia	Finland	0.002 (A)	0.006 (T)	0.015 (A:T)
Scerri et al. (81)[b]	Dyslexia	U.K.	n/s	0.0076 (G)	0.0140 (G:G) 0.0182 (G:T)
Wigg et al. (105)	Dyslexia	Canada[c]	0.021 (G)	n/s	0.026 (G:G)
Cope et al. (17)	Dyslexia	U.K.	n/s	n/s	n/s
Marino et al. (62)	Dyslexia	Italy	n/s	n/s	n/s
Meng et al. (65)	Dyslexia	U.S.[c]	n/s	n/s	n/t
Bellini et al. (4)	Dyslexia	Italy	n/s	n/s	n/t
Ylisaukko-Oja et al. (110)	Autism	Finland	n/s	n/s	n/s
Wigg et al. (106)	ADHD	Canada[c]	n/s	n/s	n/t

Paracchini et al, ARGG in press

Dyslexia candidates and brain development

- KIAA0319, DCDC2 and DYX1C1 have been implicated in neuronal migration



Joe LoTurco, University of Connecticut

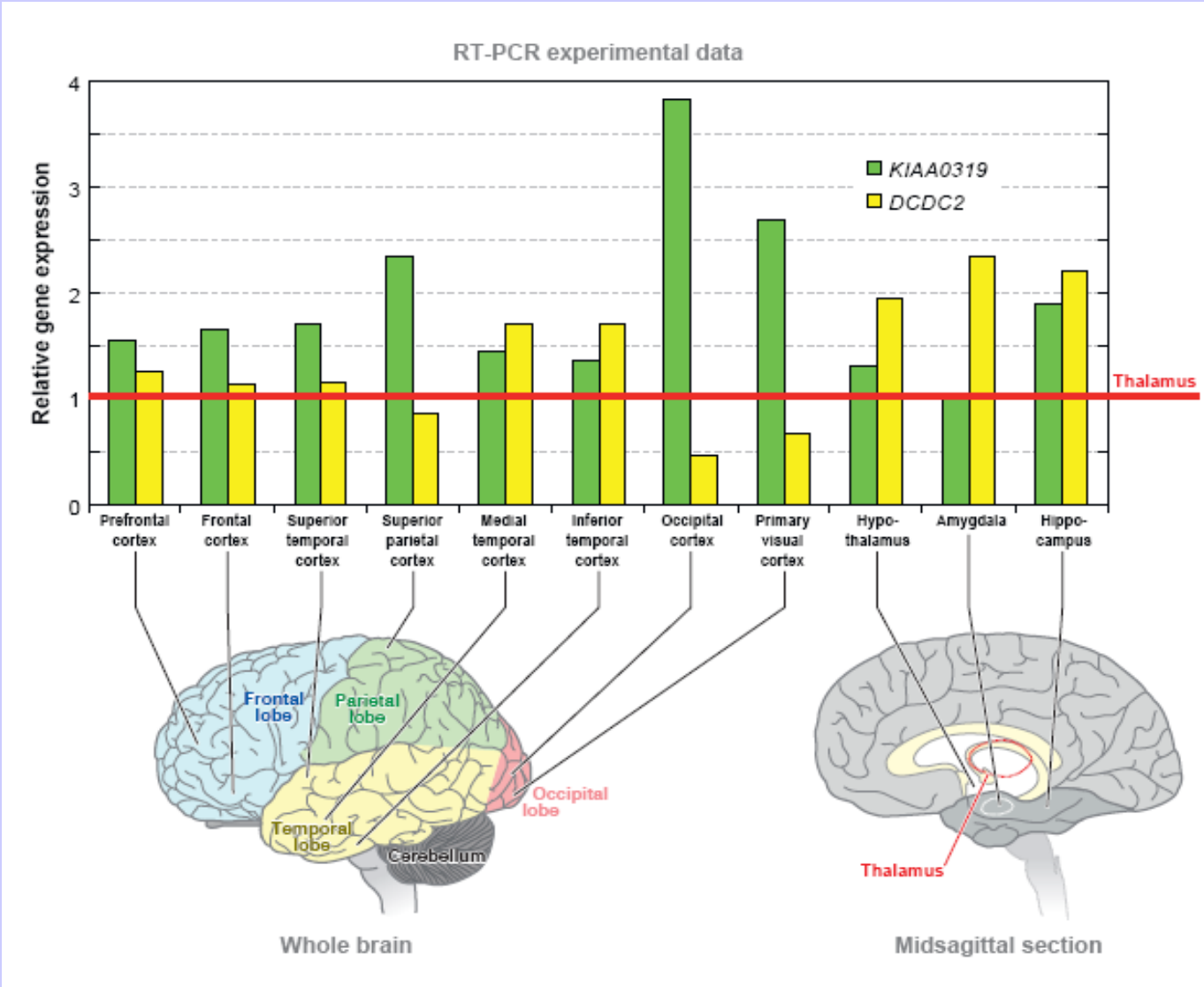
- ROBO1 is a receptor for the chemorepellent SLIT. The SLIT-ROBO system controls axon branching, commissural axon pathfinding and neuronal migration

The million dollar question

How can neuronal migration genes specifically affect reading skills?

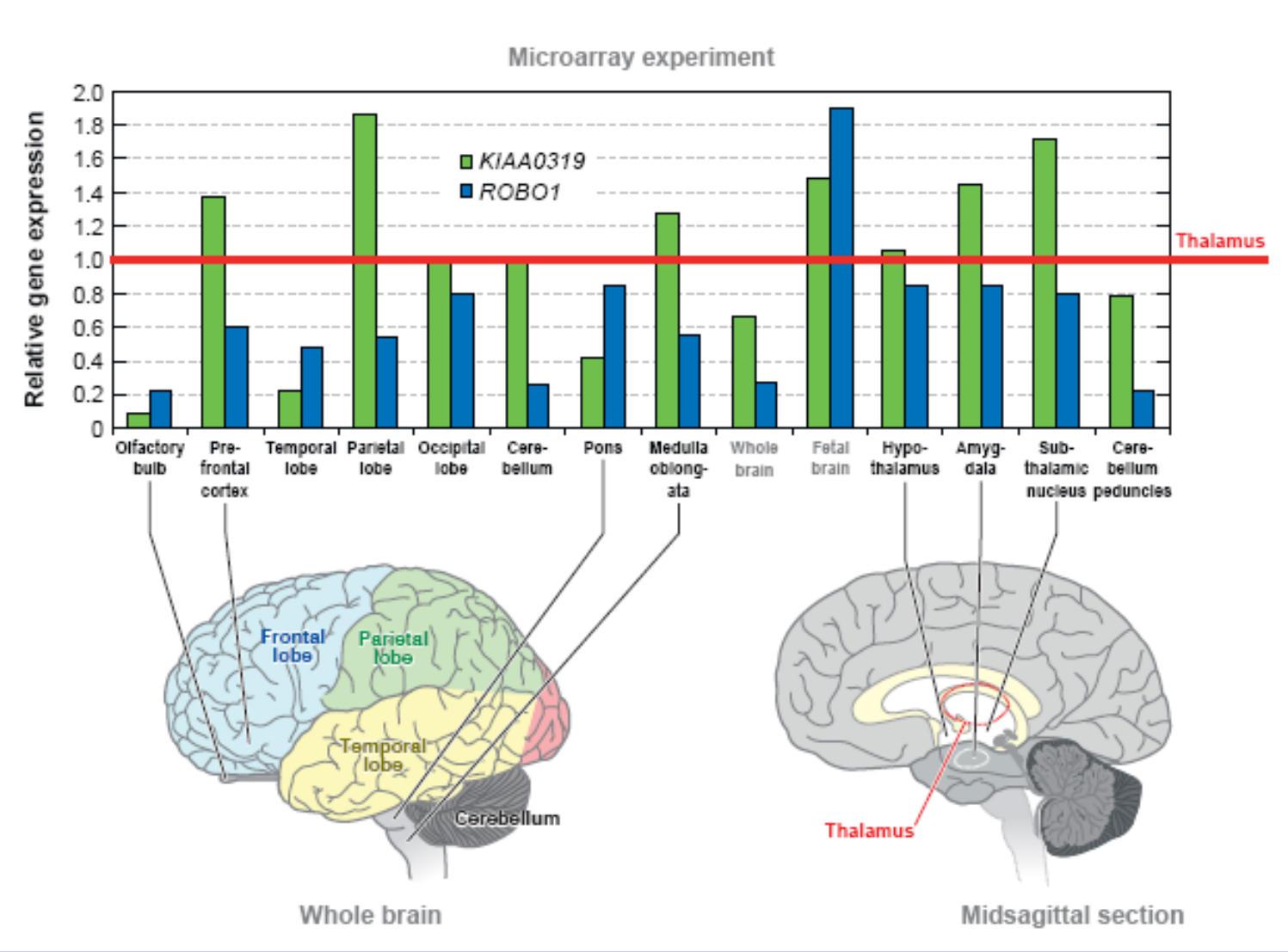
Are these genes specifically expressed in reading-related cortical regions?

Brain expression profile



Paracchini et al, ARGG in press

Brain expression profile



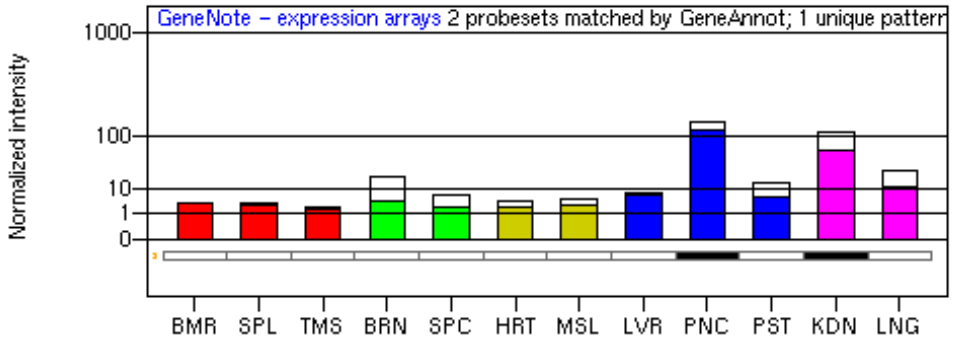
Paracchini et al, ARGG in press

EXPRESSION PROFILES IN DIFFERENT TISSUES

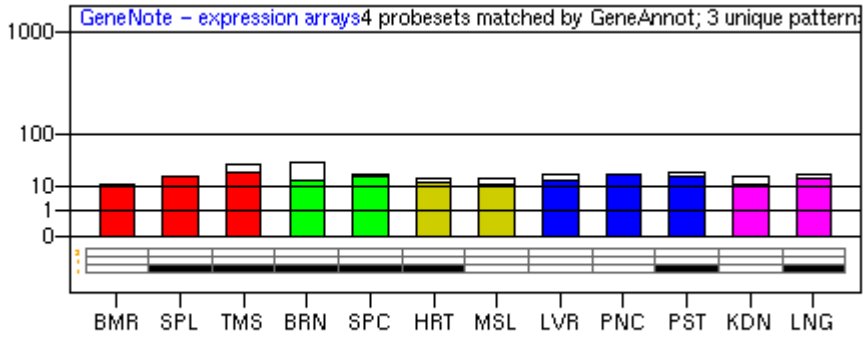
Tissue

BMR	Bone marrow
SPL	Spleen
TMS	Thymus
BRN	Brain
SPC	Spinal cord
HRT	Heart
MSL	Skeletal muscle
LVR	Liver
PNC	Pancreas
PST	Prostate
KDN	Kidney
LNG	Lung

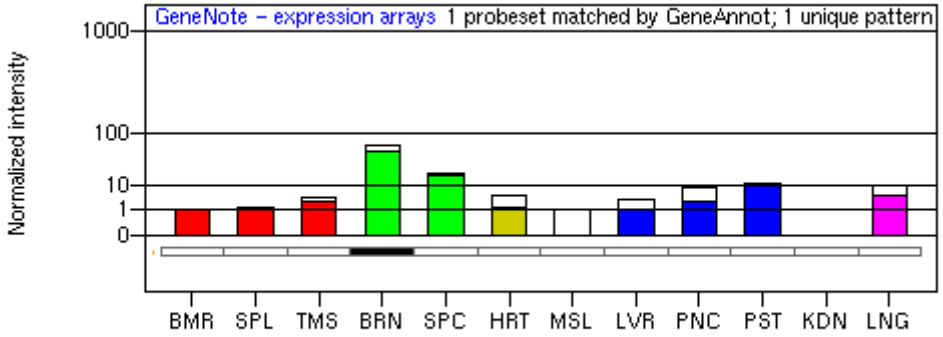
DCDC2



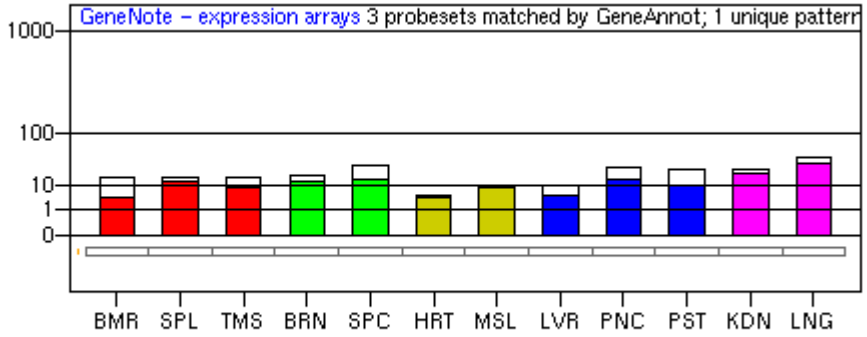
ROBO1



KIAA0319



DYX1C1



Genes and reading skills

- The candidates are not expressed in reading-specific cortical area. They are also expressed in tissues different from the brain.
- WE DON'T EXPECT TO FIND THE GENE FOR READING (as FOXP2 in not the gene for language!!)
- Suboptimal neuronal migration may result in cortical abnormalities that affect reading-related regions. The same genes may also affect other cognitive functions.
- Cortical abnormalities in specific regions would depend on multiple gene-gene, gene-environment interactions.

The NeuroDys Project

- Multidisciplinary project grouping 13 research groups from 10 European countries with different expertise
- Access to ~4000 samples
- Major goals:
  - Identify the dyslexia susceptibility genetic variants
  - Link genetic background to sub-groups of dyslexic phenotypes
  - Link genetic background to specific neurological markers

Conclusion

- The KIAA0319 gene is a strong candidate for dyslexia susceptibility, supported by association data in at least three independent samples
- A specific haplotype associated to dyslexia is also associated to reduced expression of the *KIAA0319* gene
- The *KIAA0319* is required for neuronal migration during the development of the neocortex
- The other dyslexia candidates are also involved in cortex development
- GENETICS IS PLAYING A CRUCIAL ROLE IN UNCOVERING THE CAUSES OF DYSLEXIA

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